were subjected to different IHC conditions using the C494 antibody. Different antigen retrieval techniques were applied including post-fixation, enzyme treatment alone, microwave alone and enzyme with microwave in combination. The amount of cytoplasmic staining was quantified on the CAS 2.0 cell measurement program to determine which combination of treatments yielded the best signal-to-noise ratio. Tissues undergoing no antigen retrieval and with post-fixation show good positivity in the KB V-1 cell lines for all fixation times. However, the KB 3-1 cell line is slightly positive at 4 h of fixation and becomes increasingly more positive with longer fixation time. Antigen retrieval including microwave alone, and trypsin plus microwave in combination, accentuate this effect. Without post-fixation, we again noted increasing positivity in the KB 3-1 cell line with lengthening fixation times. Microwaving decreases this effect on KB 3-1 but also decreases the signal in the KB V-1 at shorter fixation times. A combination of trypsin and microwave without post-fixation yields the best signal in the KB V-1 with minimal noise in the KB 3-1 cell line of all techniques. Formalin fixation can significantly effect immunohistochemistry results for P-gp. For ideally fixed tissues (4 h or less) individual antigen retrieval techniques do not improve the immunosignal. However, trypsin combined with microwaving without post-fixation may optimize signal to noise in these cell lines. An effort to determine the fixation history of clinical specimens submitted for IHC with consequent modification of IHC methods is critical to ensure the appropriate antigen retrieval combination and meaningful results.

## 24
## Exression of multidrug resistance (MDR1) gene in human normal tissues and head and neck squamous cell carcinomas (HNSCC)

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Various mechanisms are involved in drug resistance of tumor cells. One such mechanism is the overexpression of MDR1 gene product P-glycoprotein (P-gp) which functions as an energy dependent drug efflux pump. Using peroxidase-antiperoxidase (PAP) immunohistochemistry technique and four anti-P-gp mAbs (UIC2, gift from Drs Mechetner and Roninson; JSB-1, Bio/Can America, HYB-241, Hybritech; C219, Centocor) directed against different epitopes of the molecule, we examined the distribution of P-gp in normal human tissues and HNSCC. Under the experimental conditions only UIC2 demonstrated positive staining for P-gp in formaldehyde-fixed and paraffin-embedded tissues. Therefore, subsequent experiments were performed on frozen tissue sections. All four antibodies detected P-gp in bronchial cells, mammary ductal epithelium, gall bladder epithelium, epithelia of small and large intestine, bile canaliculi, dermal sweat glands, proximal tubules of kidney, endometrium, trophoblasts, adrenal gland, and capillaries of central nervous system, testis and papillary dermis. Presence of P-gp in capillaries of brain and testis may explain the inability of many drugs to enter these organs. With the exception of HYB-241 the antibodies always cross-reacted with myocordium and skeletal muscle. Of the 23 HNSCC that were examined, about 60% had detectable P-gp. The reaction pattern differed slightly for all four mAbs. C219 showed the most frequent reactivity. It is possible that differences noticed between antibodies are due to cross-reactivity to proteins unrelated to MDR1. Care must be taken in interpreting staining results when only one or two mAbs are used.

## 25
## Microspectrofluorimetric determination of P-gp-mediated cellular verapamil efflux using needle biopsy tumor specimens

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A new method for the functional measurement of P-glycoprotein (P-gp) mediated transport in small tumor biopsies, as an important prerequisite of clinical MDR modulator trials, was developed in the present study. Fine needle aspirates from eight patients with adenocarcinoma of the pancreas and seven patients with hepatoma were obtained under ultrasound or computer tomography guidance. The specimens were dissociated using collagenase and coated covalently to glass cover slips. Efflux of the fluorescent P-gp substrate Bodipy-verapamil (Molecular Probes, OR) from cell clusters was monitored microspectrofluorimetrically in a perfusion chamber using the Photan software (Zeiss, Oberkochen, Germany). Relative efflux rates (decrease in fluorescence intensity for the first 300 s) ranged from $-0.0$ to $-0.15 \times 10^{-2}$ s and decreased in response to perfusion with chemomodulators. Treatment of the patients consisted of oral D-verapamil (D-VPM; Knoll AG, Ludwigshafen, Germany) given at 300 mg every 6 h for 3 days (increased to 4 x 350 mg/day if tolerated), GM-CSF and doxorubicin (75 mg/m$^2$; epirubicin for pancreatic carcinoma) administered by intravenous bolus injection on day 2 and repeated every 3–4 weeks. The percentage of extruded dye (2000 s) and initial efflux velocity (linear approximation for the first 300 s) was investigated for patients with adenocarcinoma of the pancreas or hepatoma showing stable disease or progression respectively. The percentage of extruded dye was significantly higher in patients with adenocarcinoma of the pancreas which subsequently responded to therapy compared with the non-responders ($49.0 \pm 16\%$ versus $8.3 \pm 5.3\%$; both groups, $n = 4$; $p < 0.01$), no significant difference was